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The Immunobiology of Cancer Immunosurveillance and Immunoediting

Review

Gavin P. Dunn,[1] Lloyd J. Old,[2] and Robert D. Schreiber[1,*]
[1]Department of Pathology and Immunology
Center for Immunology
Washington University School of Medicine
660 South Euclid Avenue
Box 8118
St. Louis, Missouri 63110
[2]Ludwig Institute for Cancer Research
New York Branch at Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10021

The last fifteen years have seen a reemergence of interest in cancer immunosurveillance and a broadening of this concept into one termed cancer immunoediting. The latter, supported by strong experimental data derived from murine tumor models and provocative correlative data obtained by studying human cancer, holds that the immune system not only protects the host against development of primary nonviral cancers but also sculpts tumor immunogenicity. Cancer immunoediting is a process consisting of three phases: elimination (i.e., cancer immunosurveillance), equilibrium, and escape. Herein, we summarize the data supporting the existence of each of the three cancer immunoediting phases. The full understanding of the immunobiology of cancer immunosurveillance and immunoediting will hopefully stimulate development of more effective immunotherapeutic approaches to control and/or eliminate human cancers.

Comments made decades ago by the architects of the cancer immunosurveillance hypothesis, Burnet and Thomas, that "there is little ground for optimism about cancer" (Burnet, 1957) and "the greatest trouble with the idea of immunosurveillance is that it cannot be shown to exist in experimental animals" (Thomas, 1982), reflect the problems that, until recently, fomented intense debate over whether natural immune defense mechanisms can protect the host against the development of cancers of nonviral origin. The difficulty was clear: if immunosurveillance of developing tumors in immunocompetent hosts was indeed successful, then how could such an apparently invisible process be experimentally revealed? With the development of mouse tumor models using inbred mice with molecularly defined immunodeficiencies, it has become possible to demonstrate the existence of a cancer immunosurveillance process that can prevent primary tumor growth. Moreover, there is an emerging recognition that cancer immunosurveillance represents only one step of a broader process, termed cancer immunoediting, that stresses the dual host-protective versus tumor-sculpting actions of the immune system in cancer (Shankaran et al., 2001; Dunn et al., 2002, 2004). Herein, we summarize recent work on the cancer immunosurveillance and immunoediting processes—underscoring a new optimism that an enhanced understanding of naturally occurring immune system/tumor interactions will lead to the development of more effective immunologically based cancer therapies.

*Correspondence: schreiber@immunology.wustl.edu

From Cancer Immunosurveillance to Cancer Immunoediting

In 1909, Paul Ehrlich predicted that the immune system repressed the growth of carcinomas that he envisaged would otherwise occur with great frequency (Ehrlich, 1909), thus initiating a century of contentious debate over immunologic control of neoplasia. Fifty years later, as immunologists gained an enhanced understanding of transplantation and tumor immunobiology and immunogenetics, F. Macfarlane Burnet and Lewis Thomas revisited the topic of natural immune protection against cancer. Burnet's thinking was shaped by a consideration of immune tolerance; he believed that tumor cell-specific neo-antigens could provoke an effective immunologic reaction that would eliminate developing cancers (Burnet, 1957, 1964, 1971). Alternatively, Thomas's early view was evolutionary in nature; he theorized that complex long-lived organisms must possess mechanisms to protect against neoplastic disease similar to those mediating homograft rejection (Thomas, 1959). With the functional demonstration of mouse tumor-specific antigens supporting the ideas of Ehrlich, Burnet, and Thomas (Old and Boyse, 1964), the cancer immunosurveillance hypothesis, which stated that sentinel thymus-dependent cells of the body constantly surveyed host tissues for nascently transformed cells (Burnet, 1970), gained recognition. Despite subsequent challenges to this hypothesis over the next several decades (Stutman, 1974, 1979), new studies in the 1990s—fueled by technologic advances in mouse genetics and monoclonal antibody (mAb) production—reinvigorated and ultimately validated the cancer immunosurveillance concept (Smyth et al., 2001b; Dunn et al., 2002, 2004) and expanded it to incorporate the contributions of both innate and adaptive immunity.

However, there has been a growing recognition that immunosurveillance represents only one dimension of the complex relationship between the immune system and cancer (Dunn et al., 2002, 2004; Schreiber et al., 2004). Recent work has shown that the immune system may also promote the emergence of primary tumors with reduced immunogenicity that are capable of escaping immune recognition and destruction (Shankaran et al., 2001). These findings prompted the development of the cancer immunoediting hypothesis to more broadly encompass the potential host-protective and tumor-sculpting functions of the immune system throughout tumor development (Dunn et al., 2002, 2004). Cancer immunoediting is a dynamic process composed of three phases: elimination, equilibrium, and escape (Figure 1). Elimina-

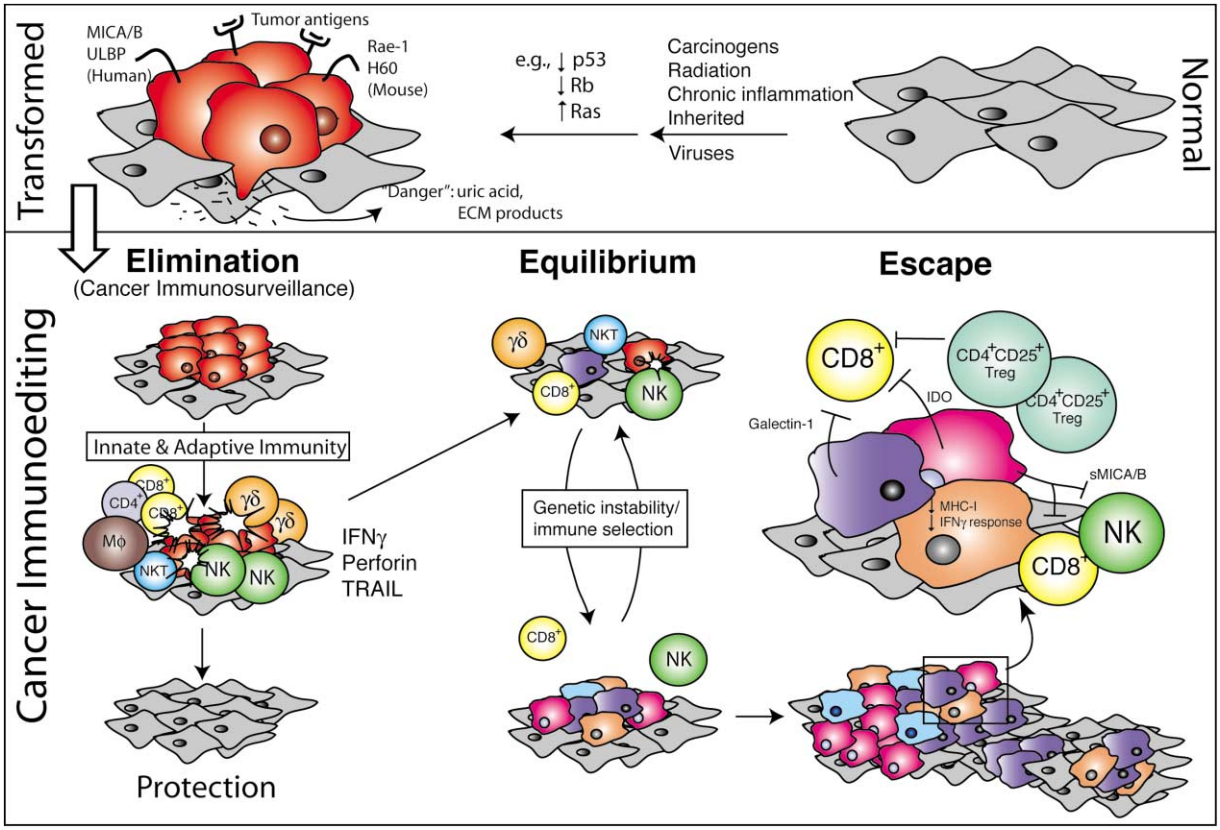

Figure 1. The Three Phases of the Cancer Immunoediting Process

Normal cells (gray) subject to common oncogenic stimuli ultimately undergo transformation and become tumor cells (red) (top). Even at early stages of tumorigenesis, these cells may express distinct tumor-specific markers and generate proinflammatory "danger" signals that initiate the cancer immunoediting process (bottom). In the first phase of elimination, cells and molecules of innate and adaptive immunity, which comprise the cancer immunosurveillance network, may eradicate the developing tumor and protect the host from tumor formation. However, if this process is not successful, the tumor cells may enter the equilibrium phase where they may be either maintained chronically or immunologically sculpted by immune "editors" to produce new populations of tumor variants. These variants may eventually evade the immune system by a variety of mechanisms and become clinically detectable in the escape phase.

tion represents the classical concept of cancer immunosurveillance, equilibrium is the period of immune-mediated latency after incomplete tumor destruction in the elimination phase, and escape refers to the final outgrowth of tumors that have outstripped immunological restraints of the equilibrium phase.

The Elimination Phase: Assembling the Cancer Immunosurveillance Network

Pivotal studies on IFN-γ (Dighe et al., 1994; Kaplan et al., 1998; Shankaran et al., 2001; Street et al., 2001, 2002) and perforin (van den Broek et al., 1996; Smyth et al., 2000a, 2000b; Street et al., 2001, 2002) have shown that deficiencies in key immunologic molecules enhanced host susceptibility to both chemically induced and spontaneous tumors, demonstrating for the first time a critical prediction of the prescient, but previously unsubstantiated, cancer immunosurveillance hypothesis. Furthermore, other compelling data suggest that immunosurveillance is not restricted to mouse models but also exists in humans (Dunn et al., 2002, 2004). Thus, today the question is not if—but, rather, how—cancer immunosurveillance functions as an extrinsic tumor suppressor and protects the immunocompetent host from the development of neoplasia. In this section, we discuss current work on the elimination phase of the cancer immunoediting process that specifically addresses the following three central questions. (1) What cells protect the host from tumor development? (2) What are the critical effector functions of the immune system in cancer immunosurveillance? (3) How does the immune system distinguish between a transformed cell and its normal progenitor?

Adaptive and Innate Immune Cells Play Critical Roles in Cancer Immunosurveillance

Rapidly accumulating data have begun to elucidate the cellular basis of cancer immunosurveillance and demonstrate that lymphocytes of both the adaptive and innate immune compartments prevent tumor development. One of the most definitive studies performed to date employed gene-targeted mice lacking the recombinase activating gene (RAG)-2 (Shankaran et al., 2001) and demonstrated that lymphocytes expressing rearranged antigen receptors play critical roles in the cancer immunosurveillance process. Mice lacking RAG-2 (or its

obligate partner RAG-1) cannot somatically rearrange lymphocyte antigen receptors and therefore cannot produce peripheral αβ T cells, B cells, NKT cells, or γδ T cells (Shinkai et al., 1992). Since RAG-2 expression is limited to cells of the lymphoid system, RAG-2$^{-/-}$ mice provided an appropriate model to exclusively study the effects of host lymphocyte deficiency on tumor development. Unlike other genetic models of immunodeficiency (such as SCID mice), the absence of RAG-2 does not affect DNA damage repair pathways in nonimmune cells undergoing transformation. Following subcutaneous injection of the chemical carcinogen 3′-methylcholanthrene (MCA), 129/SvEv RAG-2$^{-/-}$ mice developed sarcomas at the injection site faster and with greater frequency than strain-matched wild-type controls (Shankaran et al., 2001). After 160 days, 30/52 RAG-2$^{-/-}$ mice formed tumors, compared with 11/57 wild-type mice ($p < 0.0001$). Similar findings were obtained in C57BL/6 RAG-1$^{-/-}$ mice treated with MCA (Smyth et al., 2001a).

In addition, *Helicobacter*-negative RAG-2$^{-/-}$ 129/SvEv mice aged in a specific pathogen-free mouse facility and maintained on broad-spectrum antibiotics administered every other month developed significantly more spontaneous epithelial tumors than did wild-type counterparts (Shankaran et al., 2001; A.T. Bruce and R.D.S., unpublished data). Specifically, 31/32 RAG-2$^{-/-}$ mice (13–28 months old) developed spontaneous neoplasia, predominantly of the intestine. Eight had premalignant intestinal adenomas, 22 had intestinal adenocarcinomas, and one had an intestinal adenoma and a lung adenocarcinoma. In contrast, 21/33 wild-type mice remained neoplasia free to the end of their lives. Of the wild-type mice that developed neoplastic disease, six had adenomas of the Harderian gland, lung, intestine, stomach, or liver, while six others developed unrelated cancers that predominantly appeared late in life. Thus, lymphocytes protect mice against both chemically-induced and spontaneous tumor formation.

Subsequent studies have extended these findings by identifying which of the possible RAG-dependent lymphocyte subsets contribute to host antitumor defense. This work highlighted roles for γδ T cells, αβ T cells, and NKT cells in the immunosurveillance process. Mice lacking either αβ T cells (TCR β$^{-/-}$ mice) or γδ T cells (TCR δ$^{-/-}$ mice) are more susceptible to MCA-induced tumor formation than wild-type mice (on either an FVB or C57BL/6 genetic background) (Girardi et al., 2001; Gao et al., 2003). In contrast, using a carcinogenesis model involving initiation with 7, 12-dimethylbenz [*a*]anthracene (DMBA) and promotion with 12-O-tetradecanoylphorbol 13-acetate (TPA), host protection against tumor formation was found to be more dependent on the action of γδ T cells than αβ T cells. Whereas FVB strain TCR δ$^{-/-}$ mice formed significantly more DMBA/TPA tumors than wild-type FVB controls, FVB strain TCR β$^{-/-}$ did not (Girardi et al., 2001, 2003). However, TCR β$^{-/-}$ × δ$^{-/-}$ mice were significantly more susceptible to DMBA/TPA carcinogenesis than singly-deficient TCR δ$^{-/-}$ mice (Girardi et al., 2003), revealing a host-protective role for αβ T cells in the setting of γδ T cell deficiency. Interestingly, in the same study TCR δ$^{-/-}$ mice exhibited the highest ratio of carcinomas to papillomas, suggesting that γδ T cells regulate the progression of developing papillomas to more aggressive carcinomas. Together, these results reveal that αβ and γδ T cell subsets make critical, but distinct, contributions to host antitumor defense mechanisms.

NK and NKT cells also participate in cancer immunosurveillance. C57BL/6 mice, depleted of both NK and NKT cells by using the anti-NK1.1 mAb, were two to three times more susceptible to MCA-induced tumorigenesis than wild-type controls (Smyth et al., 2001a). A similar effect was observed in C57BL/6 mice treated with anti-asialo-GM1, which selectively depletes NK but not NKT cells. Although anti-asialo-GM1 can also deplete activated macrophages, the latter observation nevertheless supports the involvement of innate immune cells in blocking primary tumor development. NKT cells were also implicated in immunosurveillance by two additional observations. First, Jα281$^{-/-}$ mice, lacking Vα14Jα281-expressing invariant NKT cells, developed MCA-induced sarcomas at a higher frequency than wild-type controls (Smyth et al., 2000a). Second, mice treated with the NKT cell-activating ligand α-galactosylceramide (α-GalCer) throughout MCA-induced tumorigenesis exhibited a reduced incidence of tumors and displayed a longer latency period to tumor formation than control mice (Hayakawa et al., 2003).

A recent provocative study by Cui et al. (Cui et al., 2003) provides further evidence that innate immune cells comprise an important arm of the immunosurveillance network. A single BALB/c mouse was serendipitously found that failed to form ascites when injected intraperitoneally with the extremely aggressive S180 sarcoma cell line. Subsequent breeding revealed that the observed cancer resistance trait was germline transmissible and was likely controlled by a single autosomal dominant locus. In addition, the resulting "spontaneous regression/complete remission" (SR/CR) mice were able to kill a range of both syngeneic and allogeneic tumor cells derived from multiple tissue sites. Despite their avid antitumor resistance, SR/CR mice did not exhibit autoimmune pathology or shortened lifespan. Interestingly, resistance to S180 was also observed when the SR/CR trait was bred onto a nude genetic background, suggesting that the SR/CR phenotype is predominantly mediated by innate immune cells. Characterization of the locus controlling the SR/CR phenotype should provide new insights into innate immune control of tumor growth.

Taken together, these data not only highlight roles for both innate and adaptive immune components in the elimination phase of cancer immunoediting but also underline the complexity of the host's immune response to developing tumors. Specifically, cancer immunosurveillance appears to be a multivariable process in which immunologic responses are influenced by a tumor's cellular origin, mode of transformation, anatomic location, stromal response, cytokine production profile, and inherent immunogenicity. Thus, it remains critical to assess the effects of a wide range of immunologic components on tumor development in many different models—both chemically induced and spontaneous—to determine whether the immunosurveillance of all cancer-susceptible tissues of the body is globally similar or locally distinct.

Effector Functions Underlying Immunosurveillance: IFNγ Production and Cytotoxicity

IFNγ Production

The studies that identified physiologically relevant cellular effectors of immunosurveillance have been complemented by studies that defined two of the critical tasks these immune cells must effect to eradicate developing tumors: the production of IFN-γ and the ability to kill. Endogenously produced interferon-γ (IFN-γ) was shown to protect the host against the growth of transplanted tumors and also the formation of primary chemically induced and spontaneous tumors (Dighe et al., 1994; Kaplan et al., 1998; Shankaran et al., 2001; Street et al., 2001, 2002). Injection of neutralizing mAbs for IFN-γ into mice bearing transplanted, established Meth A tumors blocked LPS-induced tumor rejection (Dighe et al., 1994). In addition, transplanted fibrosarcomas grew faster and more efficiently in mice treated with IFN-γ-specific mAbs. In models of primary tumor formation, IFN-γ-insensitive 129/SvEv mice lacking either the IFNGR1 ligand binding subunit of the IFN-γ receptor or STAT1, the transcription factor that mediates much of IFN-γ's biologic effects on cells (Bach et al., 1997), were found to be 10–20 times more sensitive than wild-type mice to MCA tumor induction (Kaplan et al., 1998). These mice developed more tumors, more rapidly, and at lower carcinogen doses than did wild-type controls. Similar results were obtained in independent experiments by using C57BL/6 mice lacking the gene encoding IFN-γ (Street et al., 2001). In models of genetically driven tumorigenesis, mice lacking both the p53 tumor suppressor gene and either IFNGR1 or STAT1 formed a wider tumor spectrum compared to IFN-γ-sensitive mice lacking p53 only (Kaplan et al., 1998). In another study, 16/32 of IFN-$\gamma^{-/-}$ C57BL/6 mice developed disseminated lymphomas compared to 0/39 wild-type C57BL/6 mice (Street et al., 2002).

The overlap between the IFN-γ- and lymphocyte-dependent tumor suppressor pathways was revealed by comparing tumor formation in 129/SvEv mice lacking IFN-γ responsiveness (IFNGR1$^{-/-}$ or STAT1$^{-/-}$ mice), lymphocytes (RAG-2$^{-/-}$ mice), or both (RAG-2$^{-/-}$ x STAT1$^{-/-}$) (RkSk mice) (Shankaran et al., 2001). Each group of mice formed three times more chemically induced tumors than syngeneic wild-type mice when injected with 100 μg of MCA. Since no significant differences were detected between any of the gene-targeted mice, it was concluded that the IFN-γ/STAT1- and lymphocyte-dependent tumor suppressor mechanisms were largely overlapping. However, RkSk mice developed spontaneous breast tumors that were not observed in wild-type or RAG-2$^{-/-}$ mice, therefore demonstrating that the overlap between the two pathways was incomplete.

Additional work has begun to identify the relevant cellular sources and targets of IFN-γ in immunosurveillance. Recent work suggests that γδ T cells are an important source of IFN-γ during the development of protective antitumor responses (Gao et al., 2003). To directly test if γδ T cells were a physiologically important source of IFN-γ in blocking primary tumor formation, two sets of bone marrow chimeras were generated. In the control group, IFN-$\gamma^{-/-}$ mice were lethally irradiated and reconstituted with a mixture of bone marrow from TCR $\beta^{-/-}$ IFN-$\gamma^{+/+}$ and TCR $\delta^{-/-}$ IFN-$\gamma^{+/+}$mice, whereas the experimental group consisted of lethally irradiated IFN-$\gamma^{-/-}$ mice reconstituted with a mixture of bone marrow from TCR$\beta^{-/-}$ × IFN-$\gamma^{-/-}$ and TCR $\delta^{-/-}$ IFN-$\gamma^{+/+}$ mice. Thus, the only deficiency in the reconstituted experimental group was that mature γδ T cells could not produce IFN-γ. When challenged with MCA, the control bone marrow chimera group displayed a susceptibility to tumor formation similar to that of wild-type mice. In contrast, chimeric mice with γδ T cells that could not produce IFN-γ were significantly more susceptible to MCA-induced tumor formation than wild-type counterparts and displayed an increased MCA susceptibility, indistinguishable from that of IFN-$\gamma^{-/-}$ mice. Thus, γδ T cells are one physiologically relevant source of IFN-γ in the cancer immunosurveillance process. Additional work is required to identify if there are other cellular sources of IFN-γ during tumor development and to determine whether other IFN-γ-producing cells participate in responses to different types of tumors.

More is known about the physiologically relevant targets of IFN-γ's actions. Host cells are important targets of IFN-γ during development of protective antitumor immune responses. STAT1$^{-/-}$ mice with generalized IFN-γ/IFN-αβ insensitivity are more sensitive to MCA primary tumor induction (Kaplan et al., 1998) and also cannot reject highly immunogenic transplanted IFN-γ-sensitive tumor cells (Fallarino and Gajewski, 1999; V. Shankaran and R.D.S., unpublished data). At least in part, these results are explained by a requirement for IFN-γ sensitivity at the level of the host immune compartment. Through its capacity to promote the generation of tumor-specific CD4^{+} Th1 T cells and cytolytic T cells (CTL) and to activate cytocidal activity in macrophages, IFN-γ facilitates development of powerful anti-tumor effector functions mediated by both adaptive and innate immunity (Bach et al., 1997). However, the tumor cells themselves have also been shown to represent a critical cellular target of IFN-γ. Highly immunogenic MCA-induced tumor cells derived from IFN-γ sensitive RAG-2$^{-/-}$ mice are rejected when injected into naive syngeneic immunocompetent recipients (Shankaran et al., 2001; G.P.D., C.M. Koebel, and R.D.S., unpublished data). However, when the IFN-γ sensitivity of these cells is ablated by overexpression of a dominant-negative IFNGR1 mutant, they become poorly immunogenic and form aggressively growing tumors in wild-type mice (Dighe et al., 1994; G.P.D., C.M. Koebel, and R.D.S., unpublished data). Conversely, poorly immunogenic MCA-sarcoma cells derived from IFNGR1$^{-/-}$ mice become highly immunogenic cells and are rejected in immunocompetent mice after ectopic expression of wild-type IFNGR1 and restoration of IFN-γ sensitivity (Kaplan et al., 1998). IFNGR1$^{-/-}$ tumor cells are also rendered highly immunogenic by ectopic expression of IFN-γ-inducible components of the MHC class I antigen processing and presentation pathway (Shankaran et al., 2001; A.T. Bruce and R.D.S., unpublished data), thus revealing that IFN-γ's ability to upregulate tumor immunogenicity is sufficient to explain the effects on tumor detection and elimination in immunocompetent hosts.

One key remaining question is whether type I interferons (IFN-α/β) also participate in cancer immunosurveillance. Longstanding work from Gresser and colleagues

has shown that in vivo neutralization of endogenously produced IFN-α/β enhances growth of transplanted tumors in wild-type mice (Gresser and Belardelli, 2002). Moreover, administration of IFN-α/β has positive therapeutic actions on certain types of murine and human cancers (Belardelli et al., 2002). More recent in vitro work has pointed to a role for IFN-α/β in preventing cellular transformation through mechanisms involving enhanced cellular expression of the p53 tumor suppressor gene in cells exposed to type I IFN (Takaoka et al., 2003). Further work is needed to define the precise cellular targets of IFN-α/β in the cancer immunosurveillance process and to determine whether IFN-α/β functions in a manner that is identical to or distinct from IFN-γ.

Cytolytic Capacity

The second critical effector function of cancer immunosurveillance is the immune system's ability to kill tumor cells. Early studies identified perforin (pfp) as a critical cytolytic molecule in the primary host antitumor response. After challenge with MCA, $pfp^{-/-}$ mice formed two to three times more tumors than wild-type mice (van den Broek et al., 1996; Smyth et al., 2000a, 2000b; Street et al., 2001). In addition, 50% (10/20) of aging $pfp^{-/-}$ C57BL/6 mice developed spontaneous disseminated lymphomas as compared to 1/16 wild-type mice (Smyth et al., 2000b). The kinetics of lymphoma development were accelerated in $pfp^{-/-}$ mice also lacking p53 (Smyth et al., 2000b) or β2 microglobulin (Street et al., 2004).

Subsequent studies revealed an important role for the TNF-related apoptosis-inducing ligand (TRAIL) and have underscored the importance of cytotoxicity manifest by innate immunity in immunosurveillance. A member of the TNF superfamily that induces apoptosis through engagement of the TRAIL-R2 (DR5) receptor in mice, TRAIL is expressed constitutively on a subset of liver NK cells and is induced by either IFN-γ or IFN-α/β in monocytes, NK cells, and dendritic cells (Smyth et al., 2003). When injected with low doses of MCA, C57BL/6 mice treated with neutralizing antibodies to TRAIL (Takeda et al., 2002) or lacking the *TRAIL* gene (Cretney et al., 2002) developed fibrosarcomas at a higher incidence than wild-type controls. Moreover, $p53^{+/-}$ C57BL/6 mice treated with the neutralizing TRAIL-specific antibody developed more spontaneous sarcomas and disseminated lymphomas over a two-year period than control IgG-treated mice (Takeda et al., 2002). Further study is required to identify the specific innate cell subsets that manifest the TRAIL-dependent antitumor effects. Considering that the TRAIL-R2 receptor is upregulated by p53 in response to DNA damage (Wu et al., 1997, 1999), TRAIL killing may be a critical link between target cell genotoxic distress and immune-mediated destruction.

Patrolling Transformation: Mechanisms that Adaptive versus Innate Immunity Use to Distinguish Tumor Cells from Normal Cells

The third central question concerns how cells of the immunosurveillance network distinguish nascent transformed or established tumor cells from normal cells. Work over the last decade has begun to reveal the molecular basis of this crucial distinction particularly within the adaptive immune compartment. Specifically, $CD4^+$ and $CD8^+$ αβ T cells recognize tumor antigens in the context of MHC class II and class I proteins, respectively. Since the first human tumor antigen was identified in 1991 (van der Bruggen et al., 1991), many tumor antigens have been cloned and can be segregated into five categories: (1) differentiation antigens, e.g., melanocyte differentiation antigens, Melan-A/MART-1, tyrosinase, and gp-100; (2) mutational antigens, e.g., abnormal forms of p53; (3) overexpressed/amplified antigens, e.g., HER-2/neu; (4) cancer-testis (CT) antigens, e.g., MAGE and NY-ESO-1; and (5) viral antigens, e.g., EBV and HPV (Boon and van der Bruggen, 1996; Rosenberg, 1999; Old, 2003). The molecular definition of tumor antigens has revolutionized the field of tumor immunology by providing a firm basis for how the adaptive immune system discriminates between normal and neoplastic cells.

In addition to tumor antigens presented on MHC molecules, transformed cells may overexpress other molecular signposts that can function as recognition targets in the immunosurveillance process. Several studies have pointed to the NKG2D-activating receptor, expressed on NK cells, γδ T cells, and CD8 αβ T cells (Bauer et al., 1999; reviewed in Raulet, 2003), as one important component that is used by both adaptive and innate immune cells to distinguish cancer cells from normal cells. Functional NKG2D receptors complexes consist of the NKG2D ligand binding polypeptide and either the DAP10 or DAP12 signaling polypeptide (Gilfillan et al., 2002). In humans, NKG2D binds to the MHC class I chain-related proteins A and B (MICA/B), as well as the UL16 binding proteins (ULBPs) (Cosman et al., 2001; Pende et al., 2002) and the recently discovered lymphocyte effector cell toxicity-activating ligand (Letal) (Conejo-Garcia et al., 2003) (first reported as RAET1E [Radosavljevic et al., 2002] and also termed ULBP4 [Chalupny et al., 2003]). The MICA/B proteins are highly polymorphic, nonclassical MHC cell surface glycoproteins that do not associate with β2m or require TAP1 for expression (Groh et al., 1996; Bahram, 2000). Interestingly, while MIC expression in normal tissues has only been documented on the gastrointestinal epithelium of the stomach and large intestines, MICA/B proteins are often expressed in primary carcinomas of the lung, kidney, prostate, ovary, colon (Groh et al., 1999) and liver (Jinushi et al., 2003b), as well as in melanomas (Vetter et al., 2002). In addition, ULBPs (Pende et al., 2002) and Letal (Conejo-Garcia et al., 2003) are also frequently expressed on tumor cells. In mice, NKG2D binds to the retinoic acid early transcript 1 (Rae-1) family proteins Rae-1α-ε, the minor histocompatibility antigen H60 (Diefenbach et al., 2000; Cerwenka et al., 2001), and mouse UL16 binding protein-like transcript (MULT-1) (Carayannopoulos et al., 2002; Diefenbach et al., 2003). NKG2D ligand expression has been observed on a wide range of murine tumors (Diefenbach et al., 2000), and ectopic expression of Rae-1, H60, or MULT-1 was sufficient to induce the rejection of several progressively growing, transplantable tumors (Cerwenka et al., 2001; Diefenbach et al., 2001, 2003).

It will be important to characterize the regulation of NKG2D ligand expression in both human and murine cells. These molecules are often described as "stress molecules," but to date no cancer-relevant signaling

pathways have been causally linked to their expression. In human cells, MICA/B gene expression has been induced in several nontransformed human cell lines by heat shock at 42°C (Groh et al., 1999), infection with human cytomegalovirus (Groh et al., 2001), or exposure to *E. coli* (Tieng et al., 2002), although in dendritic cells MICA/B is upregulated by type I interferon (Jinushi et al., 2003a) or *M. tuberculosis* infection (Das et al., 2001). However, it remains unclear how these conditions overlap the molecular cascades that underlie neoplastic transformation (Hahn and Weinberg, 2002a; Hahn and Weinberg, 2002b). In mice, Rae-1 is upregulated by retinoic acid in F9 cells (Nomura et al., 1994) and is also expressed early in development (Raulet, 2003). In addition, one study assessed the expression of the NKG2D ligands H60 and Rae-1 after topical application of DMBA and TPA (Girardi et al., 2001). While no expression of these molecules was observed by RT-PCR in normal skin, Rae-1 and H60 expression became detectable 24 hr after carcinogen treatment. Strikingly, expression of both molecules was significantly increased in papillomas and carcinomas generated by DMBA/TPA treatment. It is possible that the transformation process itself induces molecules such as the NKG2D ligands so that the genomic upheaval of tumorigenesis is directly translated into enhanced immune recognition. Further study on the immunology of transformation will be necessary to detail when—and how—in the course of tumorigenesis a cancer cell becomes immunogenic.

One aspect of the cancer immunosurveillance process that has been the subject of much controversy is whether the unmanipulated immune system can detect a developing tumor, even one that may express distinctive recognition molecules on its surface or contain tumor-specific antigens. In the past, it was argued that cellular transformation did not provide a sufficient proinflammatory or "danger" signal to alert the immune system to the presence of a developing tumor (Matzinger, 1994; Pardoll, 2003). However, it was recently realized that (1) danger signals, such as uric acid (Shi et al., 2003), may arise from the inherent biology of the tumor itself (Seong and Matzinger, 2004) and (2) induction of proinflammatory responses through the generation of potential Toll-like receptor ligands, such as heat shock proteins (Ohashi et al., 2000; Asea et al., 2002; Srivastava, 2002), or extracellular matrix derivatives, such as hyaluronic acid (Termeer et al., 2002) or heparan sulfates (Johnson et al., 2002), may share similarities to the events that underlie activation of innate immune responses to microbial pathogens (Janeway, 1989). Importantly, whereas locally controlled inflammation may be involved in initiating responses to tumors, excessive inflammation may facilitate the transformation process (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Dranoff, 2004). It is therefore important to better define how inflammation inhibits or promotes tumor development in vivo.

Immunologic Sculpting of Cancer: When Tumors Escape Immunosurveillance

Originally, cancer immunosurveillance was envisaged as a binary process: the immune system either protected the host from the development or cancer, or it did not (Burnet, 1970). Moreover, the surveillance functions of the immune system were thought to be executed only at the earliest stages of tumorigenesis. However, recent studies have started to explain what happens when tumors develop in immunocompetent hosts, as they do in individuals with cancer, and what has emerged from this is the realization that even when immunosurveillance fails, the relationship between immunity and cancer is far from over.

An appreciation of the complexity of the immune system/tumor interaction is based on work that compared the immunogenicities of tumors derived from immunocompromised versus immunocompetent mice. In one study, 17/17 tumors derived from wild-type 129/SvEv mice and 20/20 tumors derived from immunodeficient RAG-2$^{-/-}$ mice grew progressively when transplanted into RAG-2$^{-/-}$ hosts (Shankaran et al., 2001). Furthermore, all 17 wild-type tumors grew progressively when transplanted into immunocompetent recipients. However, 8/20 tumors from RAG-2$^{-/-}$ mice were rejected when transplanted into wild-type mice. These findings showed that tumors formed in an immunodeficient environment are, as a group, more immunogenic than tumors that develop in immunocompetent hosts. Similarly, MCA-induced sarcomas derived from nude (Svane et al., 1996) or SCID mice (Engel et al., 1996) were rejected more frequently than similar tumors derived from wild-type mice when transplanted into wild-type hosts. Moreover, sarcomas from Jα281$^{-/-}$ mice grew progressively when transplanted into Jα281$^{-/-}$ recipients, but their growth was significantly impaired when transplanted into wild-type mice (Smyth et al., 2000a). In addition, lymphomas from pfp$^{-/-}$ mice grew avidly when transplanted into pfp$^{-/-}$ recipients, but most were rejected when transplanted into wild-type mice (Street et al., 2002).

Together, the functional demonstration that immunity shapes tumor immunogenicity has laid the foundation for the development of the cancer immunoediting hypothesis. This concept not only incorporates the original notion of cancer immunosurveillance but also recognizes that even after escaping immunosurveillance, a tumor's immunogenic phenotype is continuously shaped by the immunological forces in its environment. In trying to understand how tumors may ultimately be sculpted by immunity, we propose that a tumor that has breached the elimination phase of the immunoediting process may experience two subsequent phases in its interactions with the host's immune system: equilibrium, followed by escape (Dunn et al., 2002, 2004).

The Equilibrium Phase of Cancer Immunoediting

Based on the experimental systems described above, cancer immunosurveillance—i.e., the elimination phase of the cancer immunoediting process—can eradicate a significant percentage of transformed cells. However, some tumor cells may withstand the formidable pressure exerted by cancer immunosurveillance's arsenal. Therefore, we envision that there exists a period of latency extending from the end of the elimination phase to the beginning of the escape phase and the emergence of clinically detectable malignant disease. This potentially protracted period in the course of the immune

system/tumor interaction that probably occurs prior to the detection of clinically apparent tumors constitutes the equilibrium phase. The events that occur in the equilibrium phase of cancer immunoediting are likely quite similar to those previously envisaged to occur in a process termed tumor dormancy (Wheelock et al., 1981; Uhr et al., 1991). In both cases, while the immune system initially constrains the growth of heterogeneous tumors composed of unstable and rapidly mutating cells, it may approach an asymptote that Darwin could have predicted; although many of the original tumor cells are destroyed, new variants arise carrying more mutations that provide them with increased resistance to immune attack. Ultimately, the dynamic interaction between immunity and cancer in the equilibrium phase produces new populations of tumor cells vetted for survival in the immunocompetent host.

The tumor substrate on which immune cells continuously act can contain cancer cells that harbor thousands of mutations (Loeb, 1991; Loeb et al., 2003). The enormous plasticity of the cancer cell genome is thought to arise from several types of genetic instability, including nucleotide-excision repair instability, microsatellite instability, and chromosomal instability (Lengauer et al., 1998), the latter of which may induce gains or losses of whole chromosomes. Thus, the tumor cell's constant genomic metamorphosis may eventually give rise to new phenotypes that display reduced immunogenicity. Importantly, while the genesis of variation in tumor cell immunogenicity may be stochastic, the tumor that ultimately emerges from the equilibrium phase is instructively shaped by the repertoire of immune "editors" in its local environment and is thus conditioned to progress into the escape phase of the immunoediting process.

A clinical scenario that likely demonstrates the existence of the equilibrium phase in humans is the transmission of cancer from organ transplant donors to recipients. In these situations, transplanted organs appear grossly cancer free at harvest. While some organ donors are subsequently found to harbor disease in other anatomic sites, other transplant donors either have no clinical history of cancer or have been in durable remission from cancer prior to transplantation. One recent study reported the occurrence of metastatic melanoma 1–2 years posttransplant in two allograft recipients who had each received kidneys from the same donor (MacKie et al., 2003). Upon subsequent investigation, it was found that the donor had been treated for primary melanoma 16 years before her death, but was considered tumor free at the time of organ donation. This study, together with others in the literature (Penn, 1991, 1996; Elder et al., 1997; Suranyi et al., 1998), suggests that the pharmacologic suppression of the immune systems of these transplant recipients facilitated the rapid and progressive outgrowth of occult tumors that were maintained in the equilibrium phase by the donor's intact immune system.

We stress here that the cancer immunoediting process may not always represent the linear progression of a tumor from the elimination phase through the equilibrium phase and into the final escape phase of clinical detection. Indeed, this process may be terminated in the elimination phase if the cancer immunosurveillance process is successful at destroying a developing tumor. Likewise, we envision three possible outcomes for a tumor that has entered the latent period of equilibrium: (1) eventual elimination by the immune system, (2) permanent maintenance in the equilibrium phase by the cellular and molecular controls of immunity, or (3) escape from immune pressure and transit to the final escape phase of the immunoediting process. Importantly, the first two phases of the cancer immunoediting process represent potential goals for immunotherapy: tumor elimination or the durable control of cancer in equilibrium. Currently, the equilibrium phase is the most hypothetical of the three phases, and more data are needed to prove its existence. Thus, it is critical to establish new tumor models that will stringently test for the existence of the equilibrium phase during primary tumor development and subsequently define the effects of experimentally controlled variable periods of equilibrium on the immunogenicities of established cancers in immunocompetent hosts.

The Escape Phase of Cancer Immunoediting

Edited tumor cells surviving the equilibrium phase of the cancer immunoediting process enter the escape phase where tumor growth proceeds unrestrained by immune pressure. To become clinically detectable in the immunocompetent host, cancer cells must circumvent both innate and adaptive immunologic defenses. The degree to which a tumor's immunogenicity is shaped by its interaction with the host immune system may be determined by the identities of the immune editors operative during the equilibrium phase. It is possible that tumor escape from each different tissue site of origin may be mechanistically distinct. It therefore follows that metastatic lesions may experience the most significant immunologic sculpting—hewn by immune pressure from both primary tissue sites of origin as well as distant sites of manifestation.

Many studies have documented that tumor escape can be a direct consequence of alterations occurring in edited tumor targets themselves. For example, some tumor cells develop direct or indirect lesions in antigen processing and presentation pathways that facilitate evasion from adaptive immune recognition. Analysis of human tumor specimens has shown that many display losses of HLA class I proteins (Algarra et al., 2000; Marincola et al., 2000). In addition, other components of this pathway, including TAP1 and the immunoproteasome subunits LMP2 and LMP7, are frequently deficient in human tumors (Seliger et al., 2000). Moreover, other lesions were identified that indirectly lead to antigen processing or presentation defects. In one study, 4/17 (25%) human lung adenocarcinoma cell lines were found to be unresponsive to IFN-γ due to the absence or abnormal function of components of the IFN-γ receptor signaling pathway (Kaplan et al., 1998). Tumor cells expressing these lesions failed to upregulate MHC class I pathway activity when exposed to IFN-γ. Similar lesions were found in other studies that reported deficiencies of IFN receptor signaling pathway components in prostate cancer (G.P.D. and R.D.S., unpublished data) and melanoma (Wong et al., 1997).

Tumor escape has also been observed as one outcome of specific immunotherapeutic approaches. In one

study, ten patients harboring metastatic melanoma received adoptively transferred T cells specific to the tumor antigens MART-1/MelanA or gp100 with adjuvant IL-2 therapy (Yee et al., 2002). Although 8/10 patients experienced stable, minor, or mixed clinical responses, 3/5 patients studied exhibited specific loss of the tumor antigen that was targeted during treatment. Similar findings were made in two other melanoma vaccine trials in which multiple tumors from individual patients undergoing vaccination with peptides from gp-100, MART-1, and tyrosinase lost expression of either the targeted melanoma antigens or all HLA molecules on which the antigens were presented (Jager et al., 1997; Khong et al., 2004). These studies clearly show that tumors may evade both naturally occurring or therapeutically induced immune responses and suggest that at least some of the dynamics underlying the cancer immunoediting process in the unmanipulated host are also at work when the three phases of cancer immunoediting are initiated therapeutically.

Other work has shown that inhibition of the protective functions of the immune system may also facilitate tumor escape. In this scenario, immunologically sculpted tumor cell variants may overproduce immunosuppressive cytokines, such as TGF-β or IL-10 (Khong and Restifo, 2002), or inhibit immune responses through other mechanisms. One study documented that soluble forms of the MIC NKG2D ligands, secreted by certain human tumors, downregulated the NKG2D receptor on immune effector cells and attenuated lymphocyte-mediated cytotoxicity (Groh et al., 2002). Other studies demonstrated that some tumor cells overproduce inhibitors of T cell responses, such as galectin-1 (Rubinstein et al., 2004) and indoleamine 2,3-dioxygenase (IDO) (Uyttenhove et al., 2003). Finally, other developing tumors suppress induction of proinflammatory danger signals through mechanisms involving activated STAT3, leading to impaired dendritic cell maturation that, in turn, provides the developing tumor with a potential mechanism to escape immune detection (Wang et al., 2003). Thus, a tumor may directly inhibit antitumor immune responses by multiple mechanisms.

Significant interest has recently focused on the premise that tumors may also facilitate the generation, activation, or function of immunosuppressive T cell populations (Terabe and Berzofsky, 2004), such as IL-13-producing NKT cells (Terabe et al., 2000) or $CD4^+CD25^+$ regulatory T cells (T regs). The latter have attracted considerable attention due to their involvement in controlling both pathologic and protective immune responses. Originally identified as a $CD4^+$ T cell subset (comprising 5%–10% of all peripheral T cells) constitutively expressing CD25 that controls the behavior of autoreactive T cells in vivo and suppresses T cell responses in vitro (Sakaguchi et al., 1995; Read et al., 1998; Thornton and Shevach, 1998), $CD4^+CD25^+$ Tregs were subsequently suggested to play important roles in inhibiting naturally occurring and therapeutically induced protective immune responses against tumors. Two studies showed that $CD4^+CD25^+$ Tregs are often responsible for the failure of naive murine hosts to eliminate transplanted tumors (Onizuka et al., 1999; Shimizu et al., 1999). Collectively, these studies documented that depletion of $CD4^+CD25^+$ Tregs by using an anti-CD25 mAb enabled mice to reject tumors that grew progressively in control mice.

These observations have stimulated interest in defining the tumor antigens recognized by Tregs and in determining whether an inhibitory T cell subset operates similarly in human cancer patients. By using SEREX analysis (Sahin et al., 1995), an expression cloning technique wherein IgG class antibodies present in the sera of tumor bearing hosts are used to identify tumor antigens, a set of normal, nonmutated proteins, including Dna J-like 2, were identified as tumor antigens. When challenged intravenously with fibrosarcoma cells, naive mice preimmunized with a subset of these SEREX-defined antigens displayed a remarkable enhancement of pulmonary metastases compared to control mice (Nishikawa et al., 2003). This effect was inhibited if Dna J-like 2-immunized mice were first depleted of $CD25^+$ cells. Moreover, permissiveness for tumor growth could be transferred to naive mice by passive transfer of $CD4^+CD25^+$ T cells derived from Dna J-like 2-immunized hosts. More striking were the subsequent observations that (1) Dna J-like 2-immunized mice developed MCA-induced primary tumors more rapidly and with greater frequency compared to control mice and (2) this effect was abrogated if the immunized mice were pretreated with a depleting CD25-specific mAb (H. Nishikawa, T. Kato, L.J.O., and H. Shiku, unpublished data). Together, these results suggest that $CD4^+CD25^+$ Tregs play an important role in suppressing protective immune responses against both primary and transplanted tumors.

Recent studies have suggested that Tregs can also be detected in a variety of human cancers. Specifically, individuals with nonsmall cell lung cancer or cancers of the ovary (Woo et al., 2001), breast, or pancreas (Liyanage et al., 2002) all displayed elevated levels of $CD4^+CD25^+$ Tregs. Moreover, another study has identified the CT antigen LAGE-1 as the first human tumor antigen specifically recognized by Tregs (Wang et al., 2004). These studies not only complement those in animal models but also point to the probable clinical relevance of regulatory T cell activity in human cancer. Further studies will be necessary to characterize the phenotypes of Tregs, elucidate the molecular basis of their suppressive functions and identify more fully their physiologic ligands.

Conclusion

In his writings about cancer immunosurveillance, Lewis Thomas reflected that, "It seemed to me then, and still does, that some such built-in immunologic mechanism *ought* to exist for natural defense against cancer" (Thomas, 1982). In this review, we have summarized the functional evidence showing that such immunologic defenses do, in fact, exist in the immunocompetent host and have discussed the nature of the known cellular and molecular components that inhibit tumor development. Furthermore, we have pointed out several remaining critical questions whose answers will ultimately frame a full understanding of the host-protective cancer immunosurveillance network. We have also discussed the studies demonstrating that cancer immunosurveillance is not the whole story, thus prompting the development of the cancer immunoediting hypothesis—i.e., the ac-

knowledgment that immunity's powers to protect the host from cancer may also drive the generation of tumors better suited to survive in an immunologically intact environment. The most significant clinical implication of this hypothesis is that most, if not all, tumors that develop in humans may have undergone immunologic sculpting as a result of a cancer immunoediting process, with the most dramatic consequences of the process probably occurring before the tumor is clinically detectable. Ultimately, if the cancer immunoediting process indeed emerges as one of the leitmotifs of cancer progression, then an improved understanding of the immunobiology of cancer immunoediting and a molecular definition of how tumors are shaped by this process will undoubtedly bring us closer to tumor immunology's capstone: the use of immunotherapy to control and/or eradicate neoplastic disease in the human cancer patient.

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